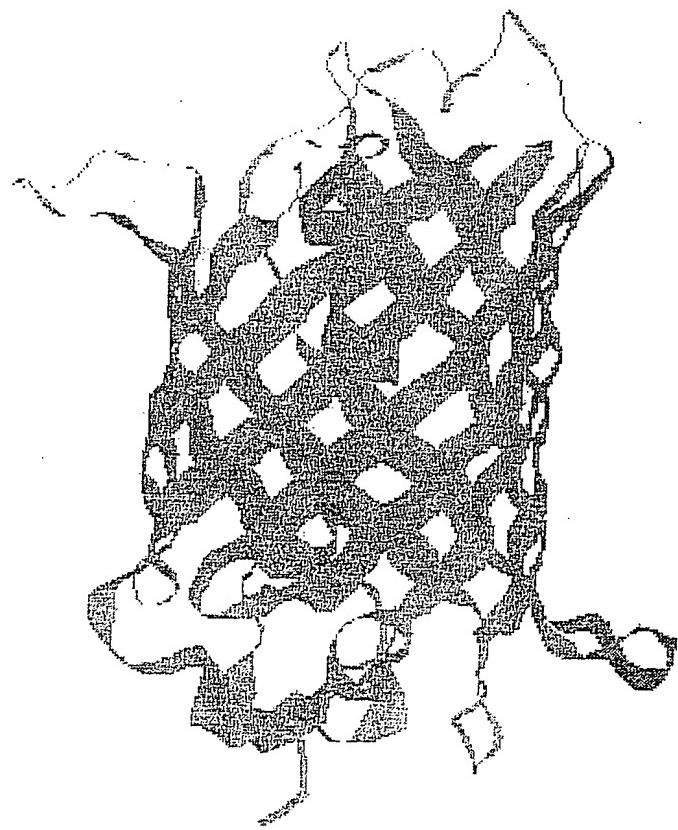


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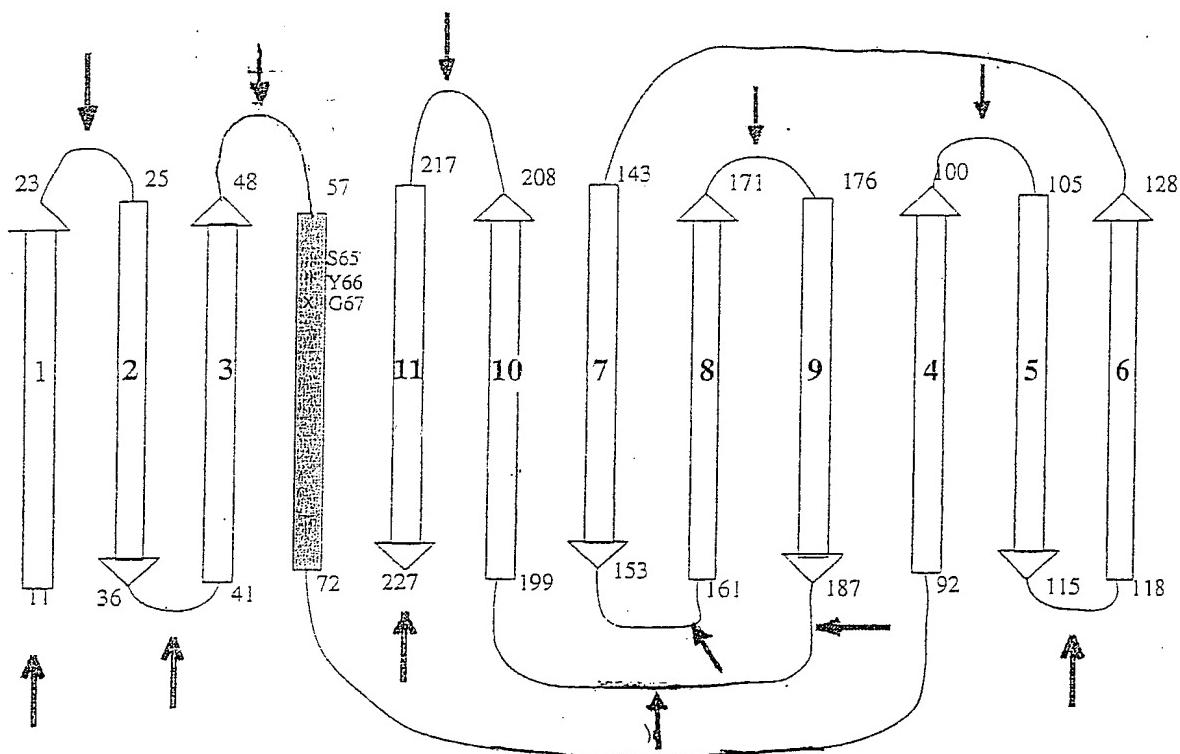


**concept** - binding to fluorescent protein  
changes fluorescence spectrum

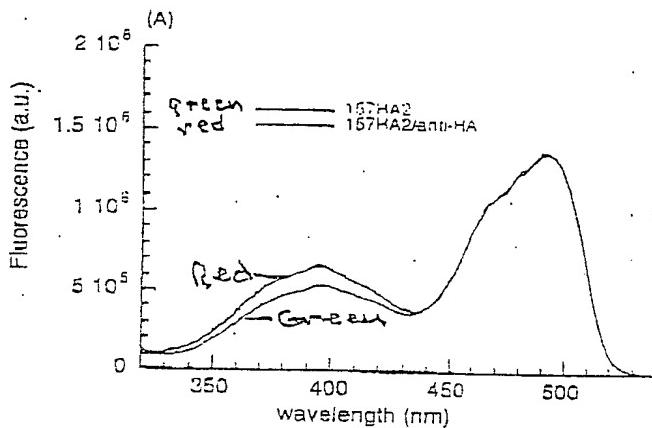
**goal** - convert the Green Fluorescent Protein  
into a universal biosensor, afFP

**Figure 1A**

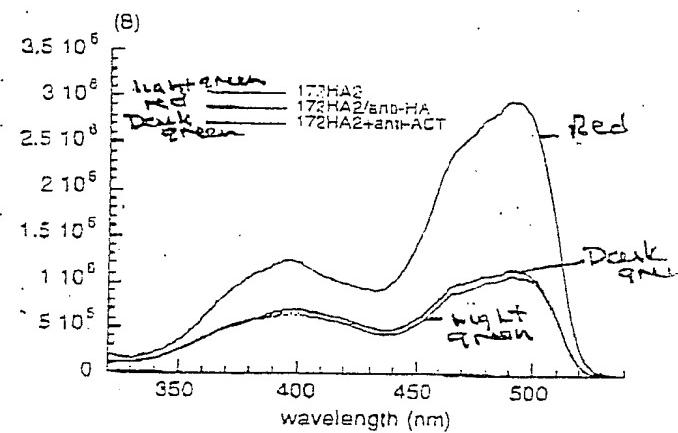
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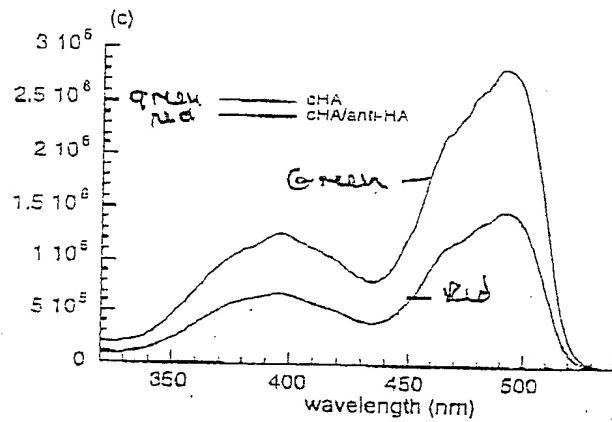
**Figure 1B**



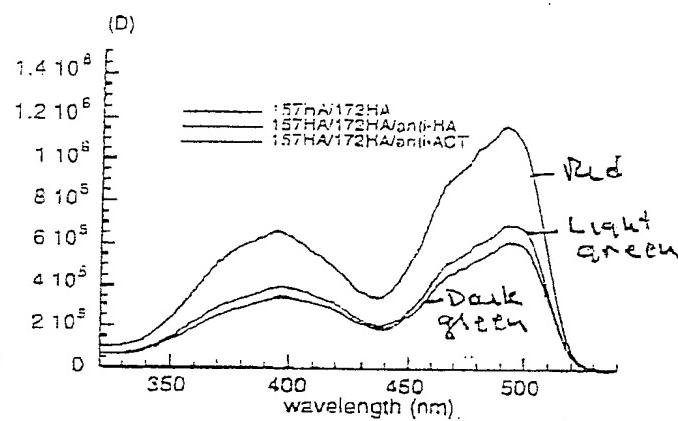
**Figure 2A**



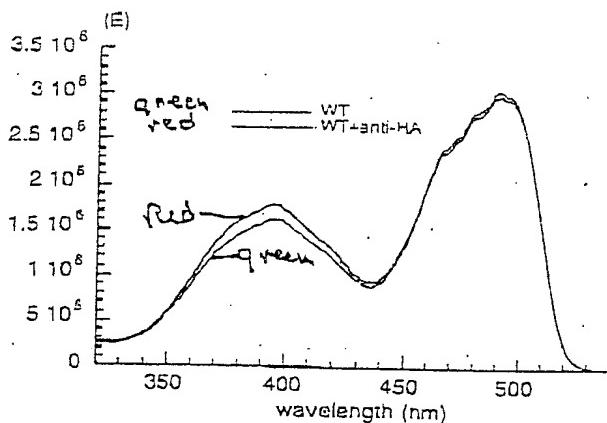
**Figure 2B**



**Figure 2C**

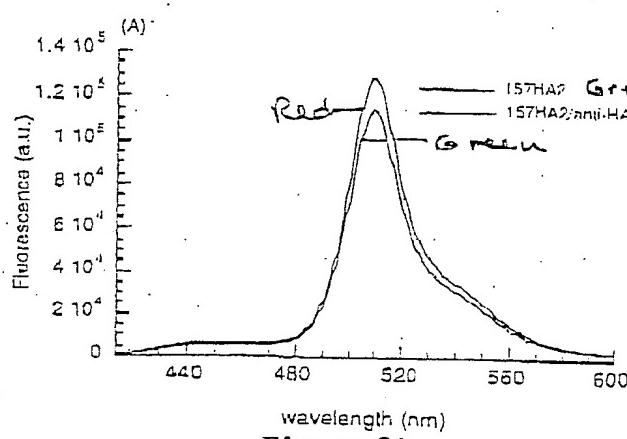


**Figure 2D**

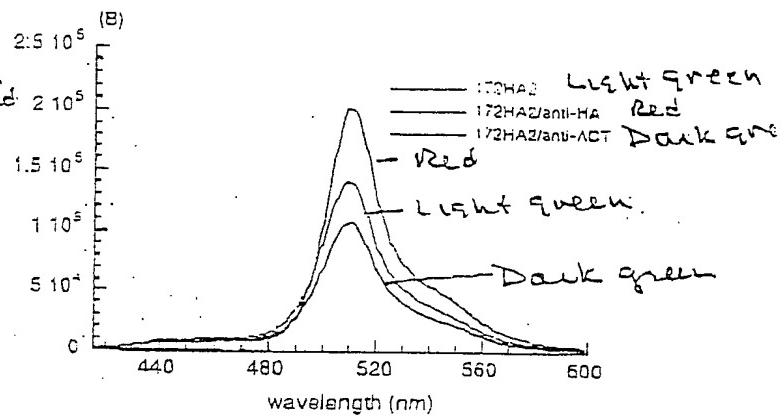


**Figure 2E**

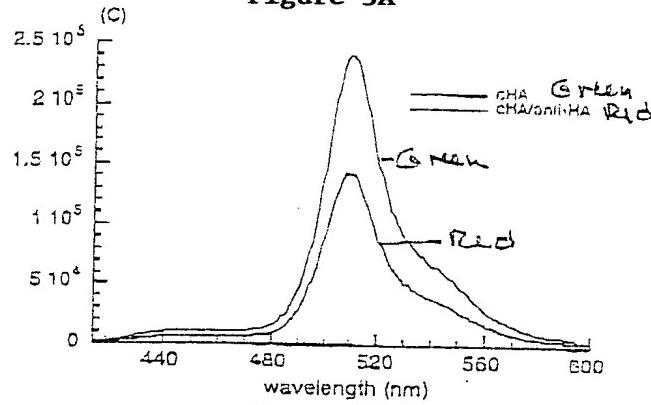
Excitation spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, and anti-ACT (monoclonal antibody against antichymotrypsin) were 0.3 mg/ml, 3.4 mg/ml, 3.4 mg/ml respectively. The spectra were collected at fixed emission wavelength of 550 nm. The red lines represented complexes. The green lines represented mutants alone. The blue lines represented mutants plus an nonspecific antibody as negative control.



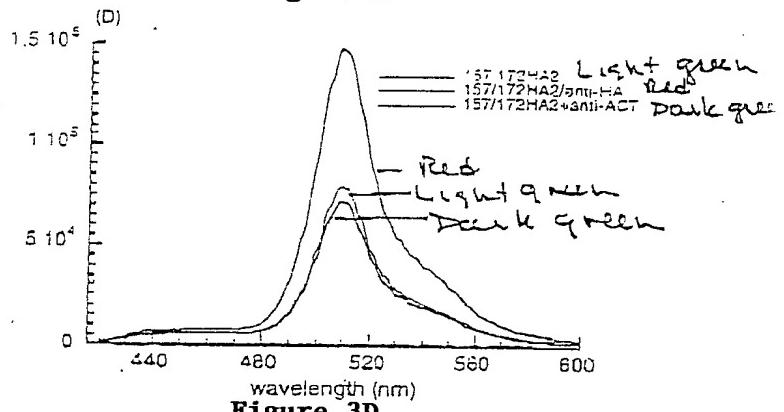
**Figure 3A**



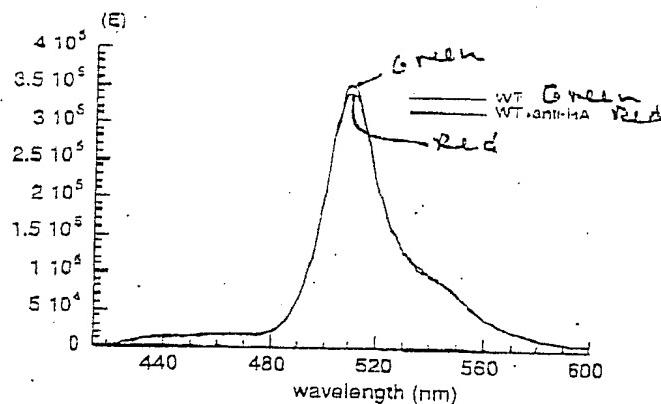
**Figure 3B**



**Figure 3C**

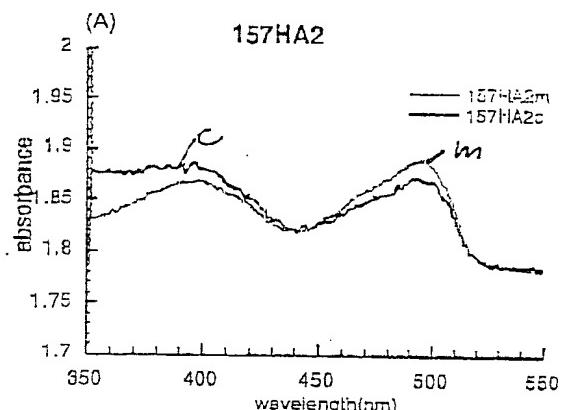


**Figure 3D**

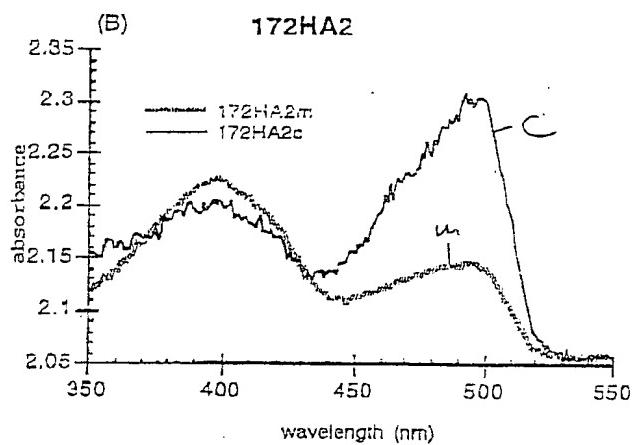


**Figure 3E**

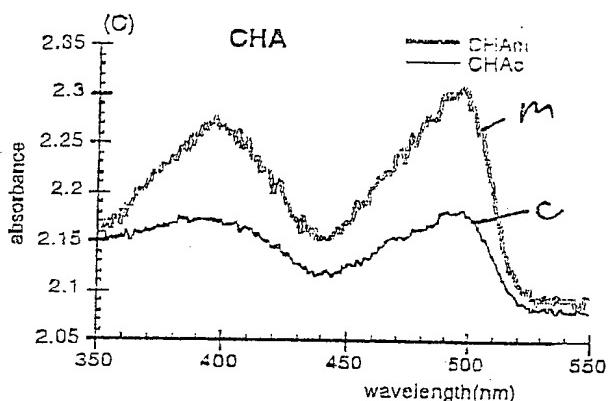
Emission spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, and anti-ACT (monoclonal antibody against antichymotrypsin) were 0.3 mg/ml, 3.4 mg/ml, 3.4 mg/ml respectively. The spectra were collected at fixed excitation wavelength of 395 nm. The red lines represented complexes. The green lines represented mutants alone. The blue lines represented mutants plus an nonspecific antibody as negative control.



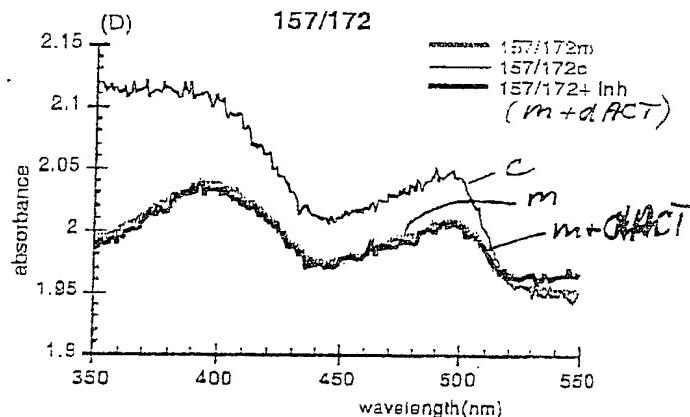
**Figure 4A**



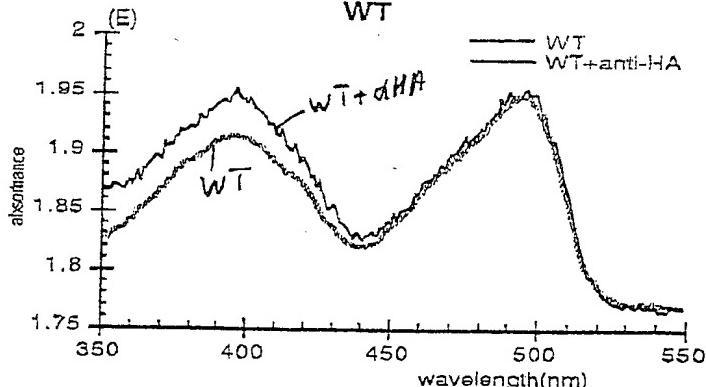
**Figure 4B**



**Figure 4C**



**Figure 4D**

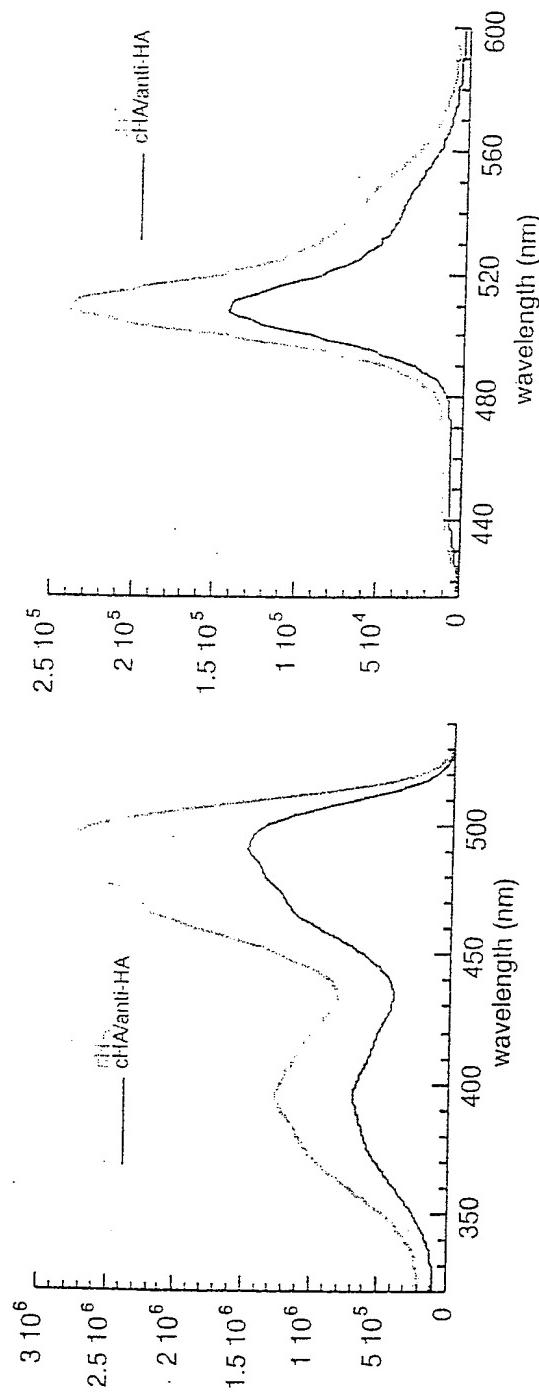


**Figure 4E**

Absorption spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, and anti-ACT (monoclonal antibody against antichymotrypsin) were 0.3 mg/ml, 3.4 mg/ml, 3.4 mg/ml respectively. The red lines represented complexes. The green lines represented mutants alone. The blue lines represented mutants plus an nonspecific antibody as negative control

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# Enhanced fluorescence upon binding



**Figure 5**

emission  
excitation

## 157HA



## 172HA



## 157HA2



## 157cyHA2

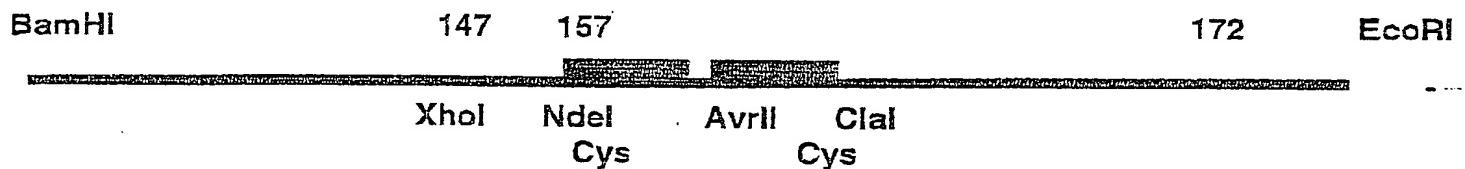


Figure 6A

## 157HA/172HA



## 172HA2



## 157HA/CHA

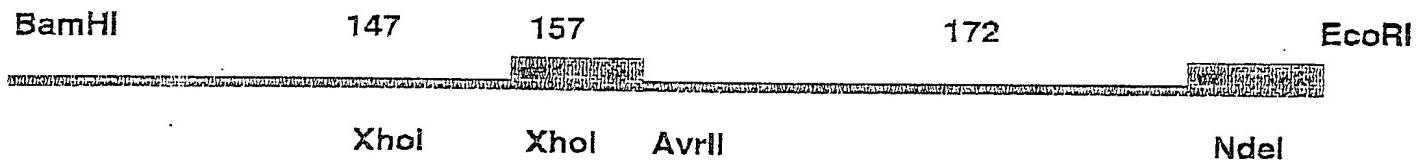
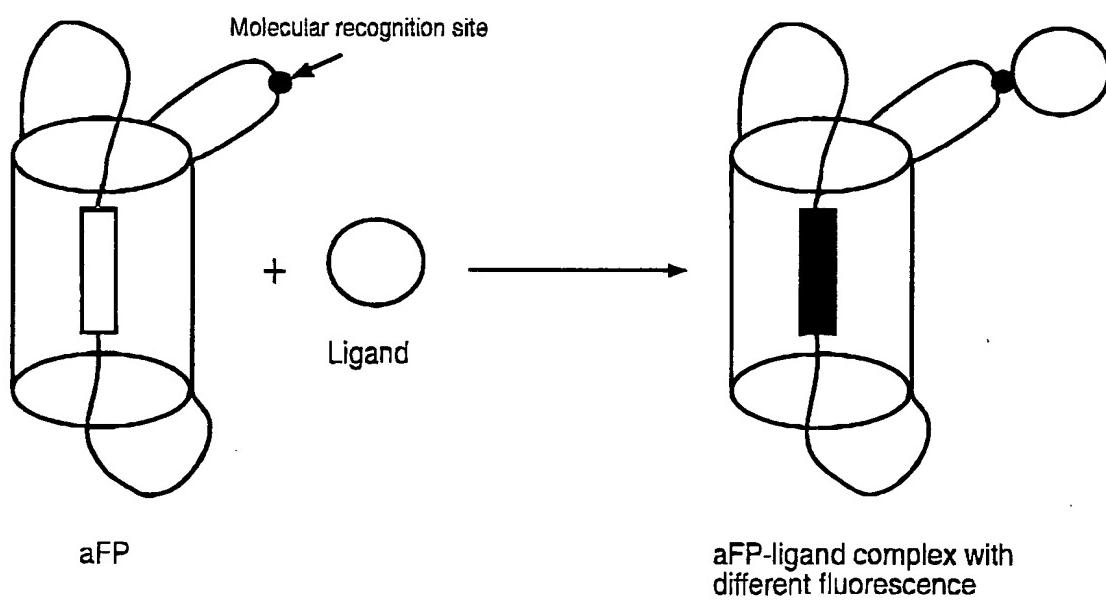


Figure 6B

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Schematic presentation of the ligand-activated affinity fluorescence biosensor derived from the GFP. The aFP is composed of one or multiple binding sequence(s) on the surface loops. Binding to the ligand may result in different fluorescence properties, such as enhanced, quenched, or wavelength shifted fluorescence.

**Figure 7**

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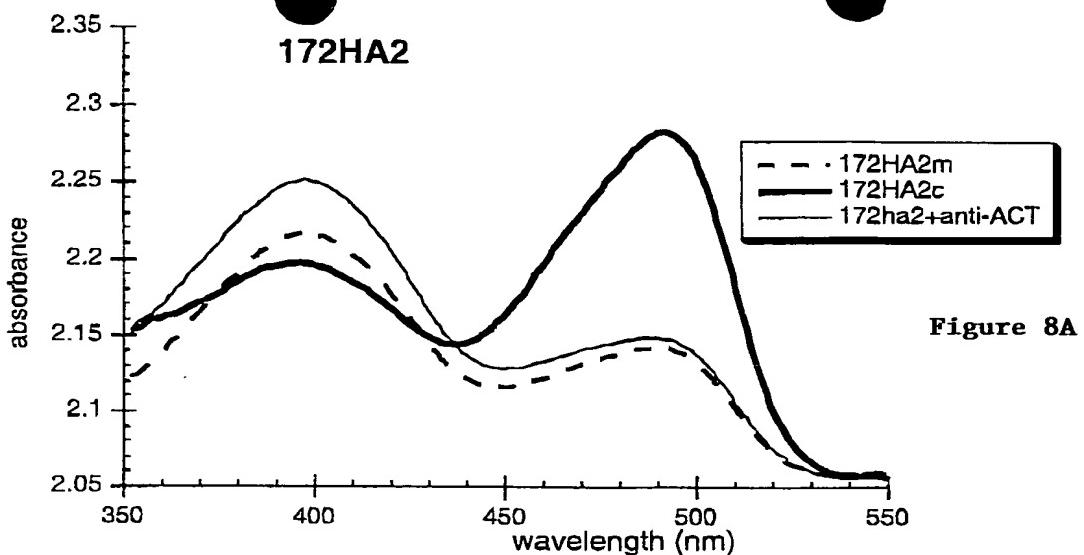


Figure 8A

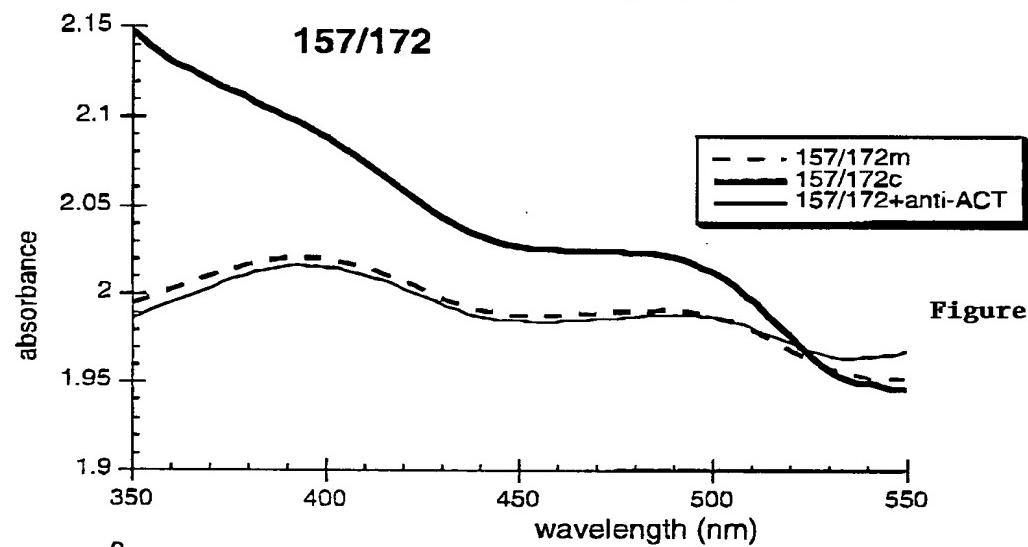


Figure 8B

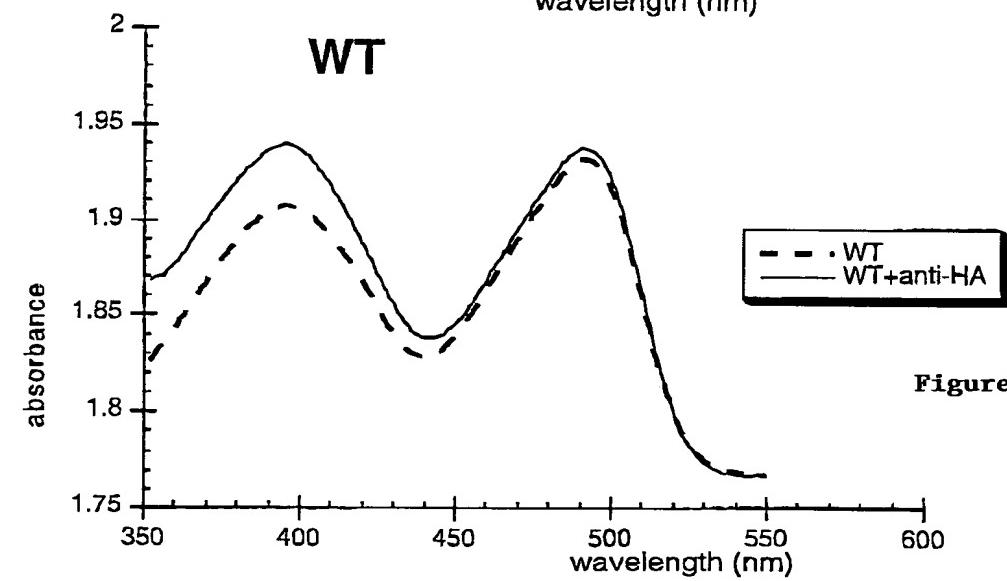


Figure 8C

Absorption spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, and anti-ACT (monoclonal antibody against antichymotrypsin) were 0.3 mg/ml, 3.4 mg/ml, 3.4 mg/ml respectively. The thick lines represented complexes. The dashed lines represented mutants alone. The thin lines represented mutants plus a nonspecific antibody as negative control

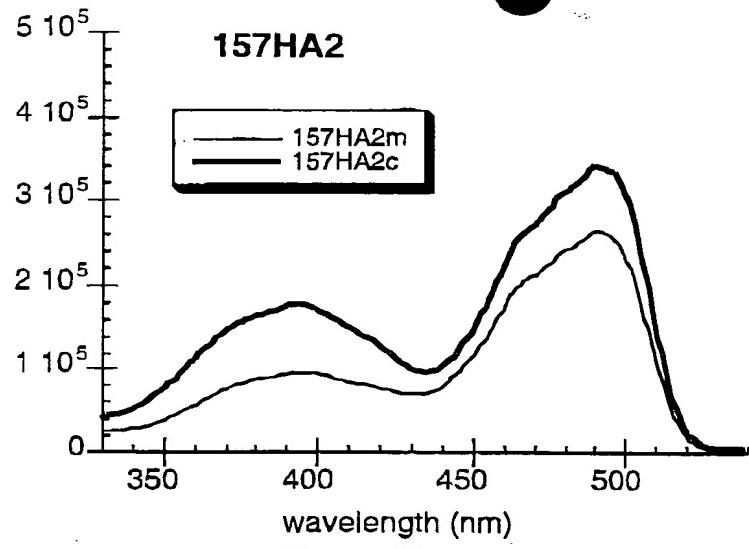


Figure 9A

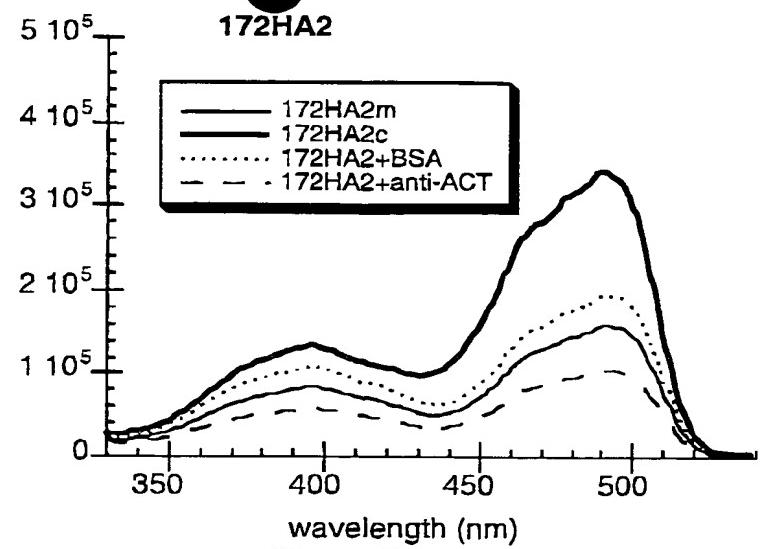


Figure 9B

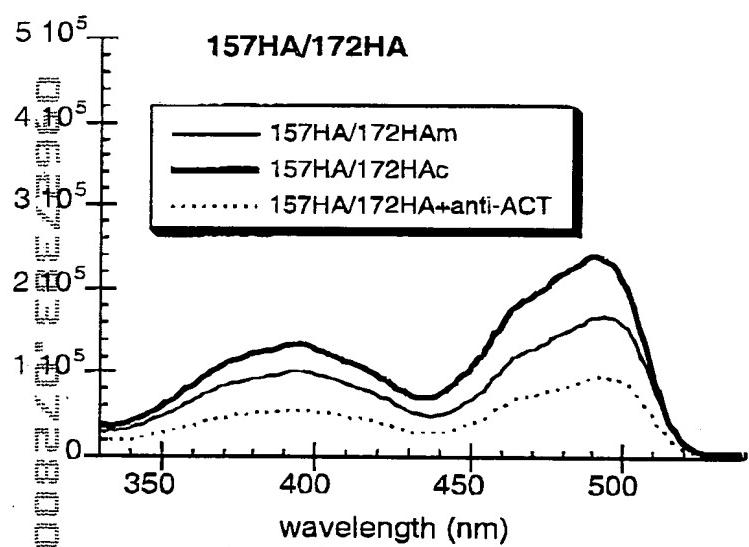


Figure 9C

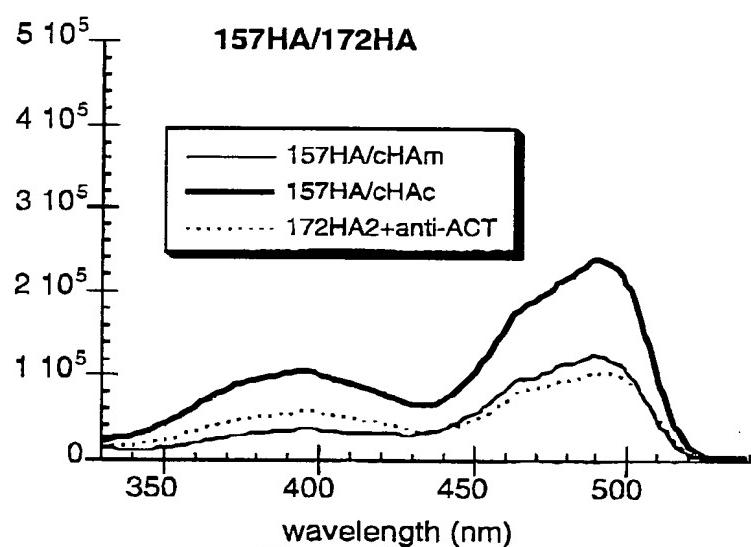


Figure 9D

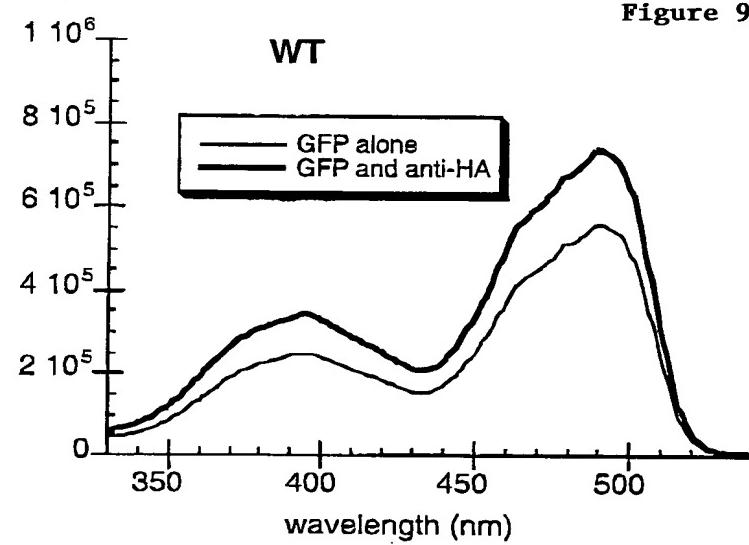


Figure 9E

Excitation spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, anti-ACT (monoclonal antibody against antichymotrypsin), and BSA were 0.025 mg/ml, 0.2 mg/ml, 0.2 mg/ml, and 0.2 mg/ml respectively. The spectra were collected at fixed emission wavelength of 550 nm. The solid thick lines represented complexes. The solid thin lines represented mutants alone. The dash lines represented mutants plus an nonspecific antibody as negative control. The dot

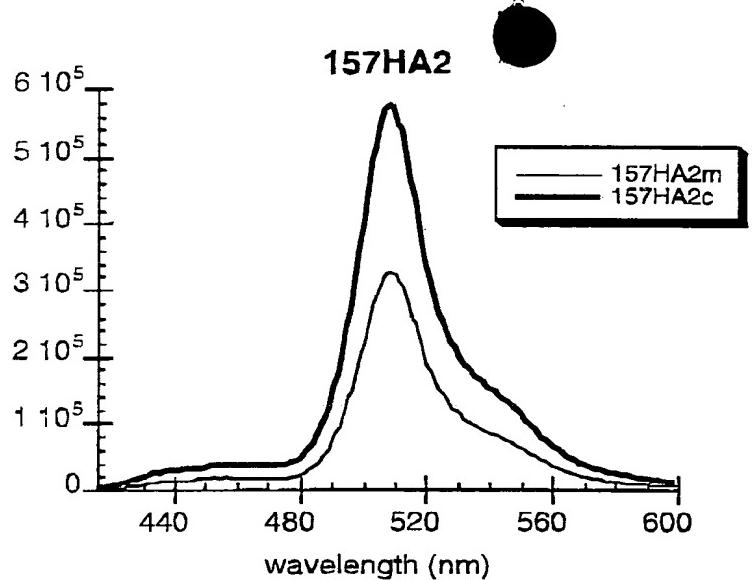


Figure 10A

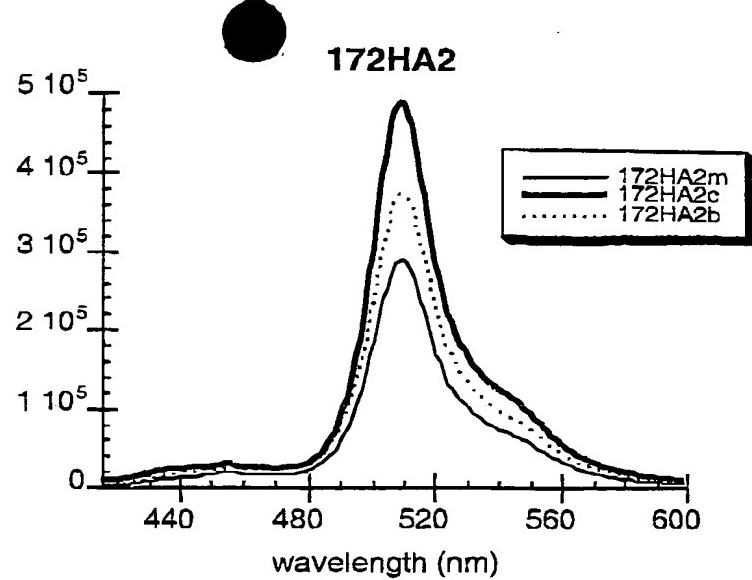


Figure 10B

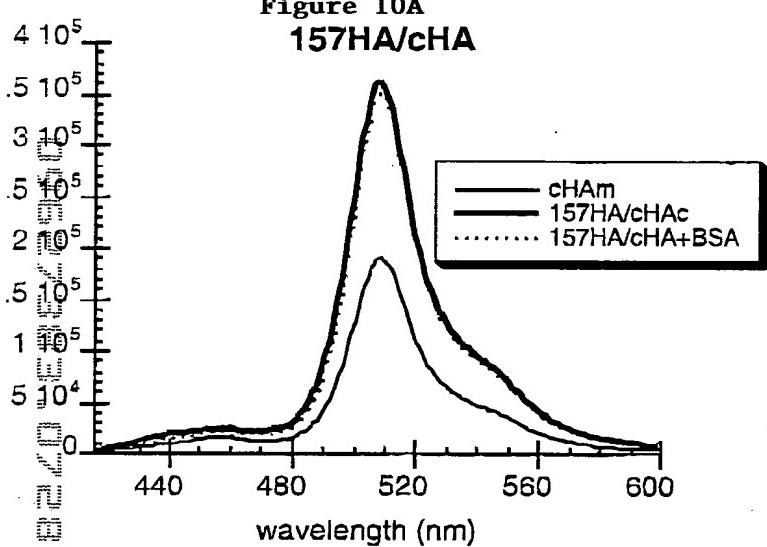


Figure 10C

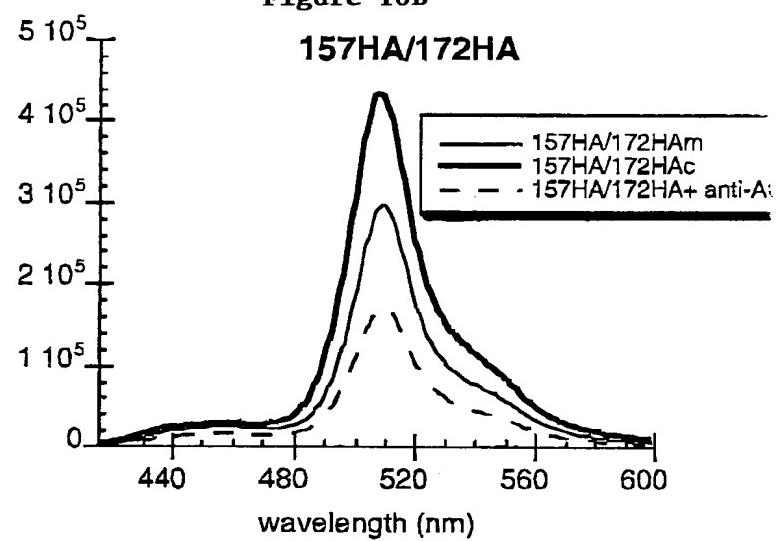


Figure 10D

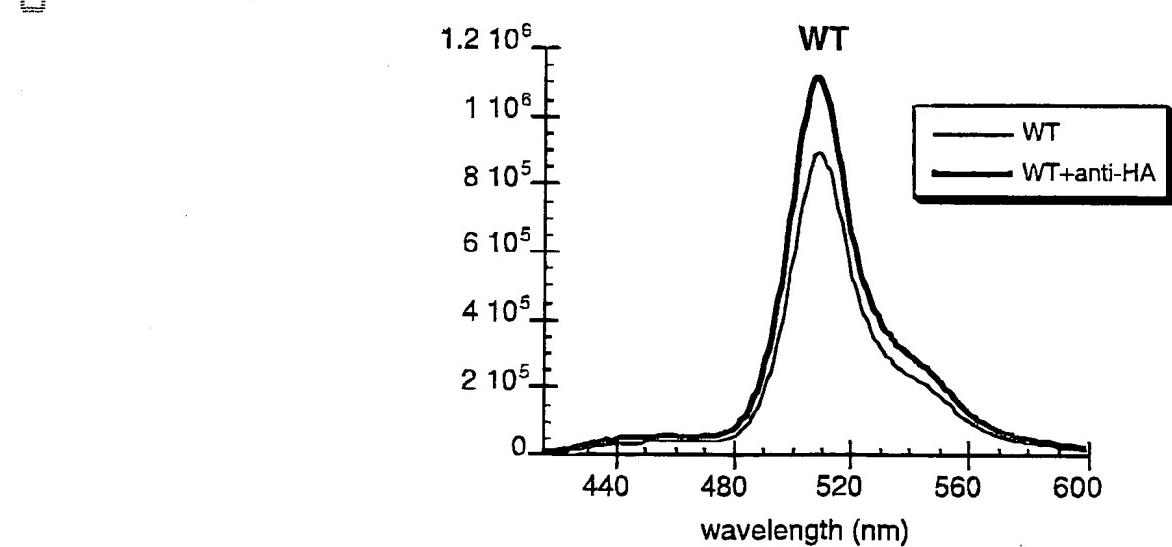
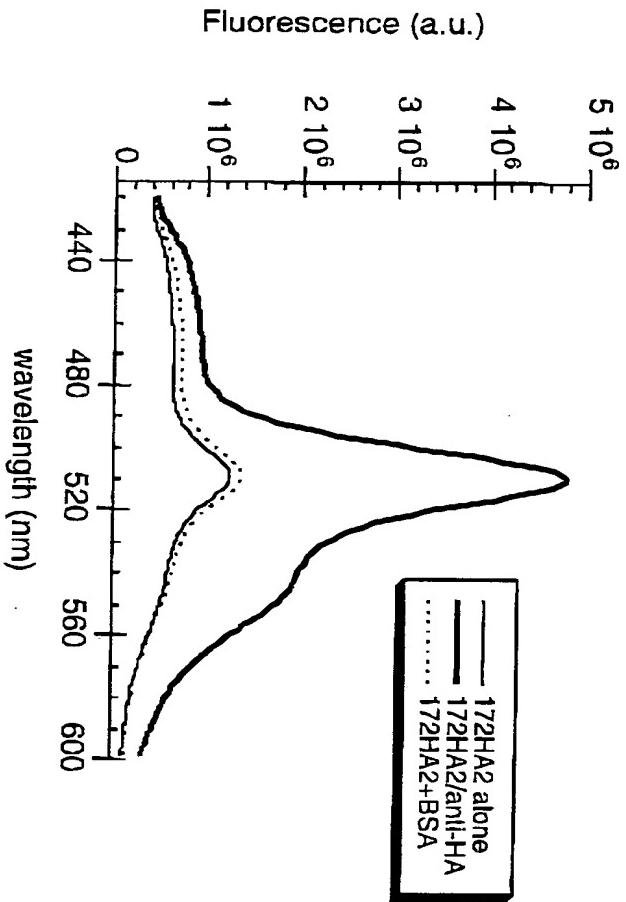


Figure 10E

Emission spectra of HA2 mutants and complexes with anti-HA. The concentrations of HA2 mutants, anti-HA, anti-ACT (monoclonal antibody against antichymotrypsin), and BSA were 0.025 mg/ml, 0.2 mg/ml, 0.2 mg/ml, and 0.2 mg/ml respectively. The spectra were collected at fixed excitation wavelength of 395 nm. The red lines represented complexes. The thick lines represented mutants alone. The dash lines represented mutants plus an nonspecific antibody as negative control. The dot lines

Figure 11



Emission spectra for the 172HA2 on the nitrocellulose membrane after wash.  
The thin line represented 172HA2 alone. The thick line represented 172HA2/anti-HA complex.  
The dot line represented 172HA2 with BSA.